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MOTH LEGS: EXCELLENT SOURCE OF TISSUE FOR DNA EXTRACTION (LEPIDOPTERA:NOCTUIDAE)

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ABSTRACT

Recent advances in taxonomy utilize molecular tools for identification & classification and to study genetic correlation between organisms. Different nuclear and mitochondrial genetic markers are utilized for this purpose. DNA isolation is initial and important step for such studies. Though, practically any tissue can be used for DNA isolation, present study suggests that legs serve as excellent source of tissue for DNA isolation in moths belonging to family Noctuidae.

KEYWORDS : DNA isolation, Genetic Markers, Molecular Tools, Noctuidae

On account of extensive diversity and huge variety, Insecta holds first position in being most diverse class amongst kingdom Animalia (Chapman, 2006). With beautiful wings and vibrant coloured scales, Lepidoptera stands second after coloeoptera in diversity (Erwin and Terry, 1997). Almost 85% of lepidopteran population accounts for moths and over 1,60,00 species are seen around the world. Noctuidae is one amongst important families of order Lepidoptera having around 36 subfamilies and 4200 genera in it.

Moths have always been a difficult group for studying taxonomy owing to their great diversity and presence of many sub-species and cryptic species. For the same reason, earlier taxonomists used anatomical tools for identifying and classifying moths. But, with beginning of present century, anatomical tools are quite taken over by molecular tools. Different nuclear and mitochondrial markers (genetic markers) are used for understanding phylogeny and thus identification and classification using them. All these studies at molecular level have one thing in common i.e. use of DNA. Also, identification and classification by use of phenotypic features i.e. by observing wing pattern and colour, are genetically controlled (Gilbert et al., 1988; Nijhout, 1991). Thus, there exists great genetic diversity and in either of methods to study this diversity importance of DNA remains same. Hence, DNA isolation becomes extensively important step in all these studies.

Though practically DNA can be isolated from any tissue, selection of tissue is important as it affects the quality and quantity of DNA isolated. It is of great importance that the tissue selected for DNA isolation causes minimum damage to rest of specimen (Calderon-Cortes et al.,2010; Hundsdoerfer and Kitching, 2010; Knolke, et al., 2005) as the specimen is generally further preserved for voucher preparation and for future reference. In present study, comparison between different tissue sources was done to identify the most suitable and excellent source of DNA from seven species of moths belonging to family Noctuidae.

MATERIALS AND METHODS

Specimens

Using light traps moths were collected. Moths were identified by their morphological characters and by using identification guide into six genus and seven species (MOB). *Eudocima materna, Ophiusa tirracha, Bastilla stuposa, Eudocima srivijiyana, Serrodes campana, Erebus marcops* and *Artena dotata* were the seven species considered under study. Specimens were killed using chloroform and were spread on spreading boards followed

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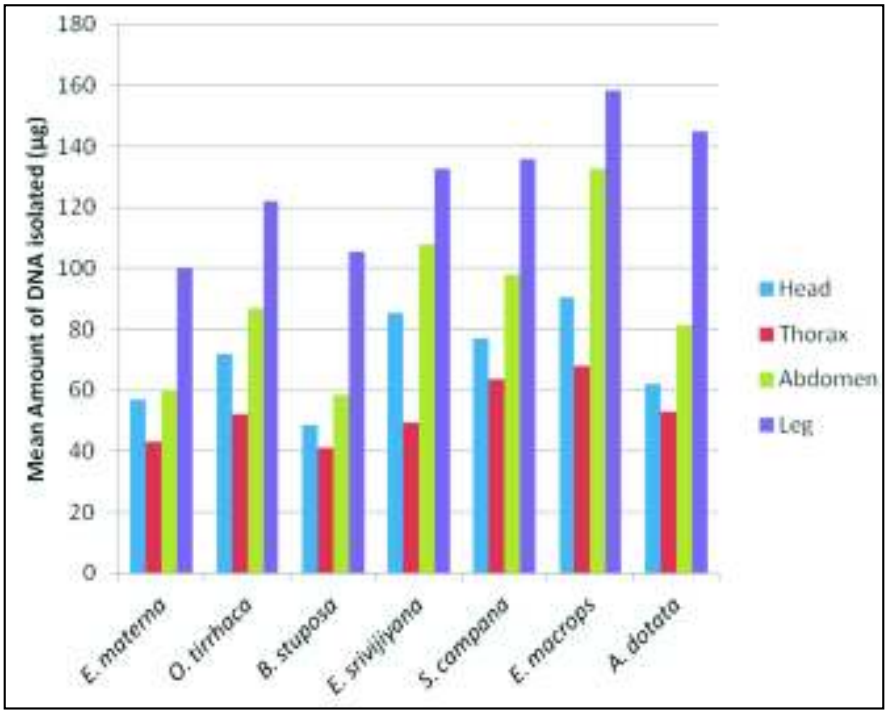

Figure :Mean amount of DNA isolated (in µg) from different body parts

by oven drying at 57°C. The preserved specimens were stored in moisture free insect boxes. All the moths were de-winged with help of sharp scissors after the identification. Four tissue sources selected were Head, Thorax, Abdomen and Legs for each specimen as all these four sources of tissue were unique in the composition and concentration of cells.

DNA Isolation

DNA was isolated from each of head, thorax, abdomen and legs by method described by Sperling et.al., (Sperling et al., 1994). DNA extraction was done using mixture of Phenol, chloroform and isoamyal alcohol (25:24:1) and DNA precipitation was done using chilled isopropanol. (Chomczynski and Sacchi, 1987; Chomczynski and Sacchi, 2006). DNA pellet was washed and purified with 70% ethanol. The purified pellet was dissolved in saline citrate solution which was further used for DNA quantification.

DNA Quantification

Colorimetric quantification of isolated DNA was performed. The extracted DNA was reacted with diphenyl amine reagent and their absorbance was noted at 600nm. The values were plotted along with standard values to find the concentration of DNA extracted from each body part.

Statistical Analysis (ANOVA)

To compare the DNA isolated from different body parts of Noctuidae moths, we proposed the null hypothesis HO as, "there is no significant difference in the amount of DNA isolated from different body parts of moths". The alternate hypothesis H_1 states that, "there is significant difference in the amount of DNA isolated from different body parts of moths".

RESULTS AND DISCUSSION

The DNA isolated in present study was from four different body parts of each individual moth. Statistical analysis was done using ANOVA to compare the quantity of DNA isolated from different tissue sources of same moth individual. It was found that in all cases calculated F ratio: *Eudocima materna* (14024.9), *Ophiusa tirracha* (20227.3), *Bastilla stuposa* (7624.92), *Eudocima srivijiyana* (12728.4), *Serrodes campana* (22337.3), *Erebus marcops* (8510.07) and *Artena dotata* (13843.5) was greater than the table F ratio (3.24), suggesting that the amount of DNA isolated from different body parts is variable and shows significant difference in all species under study.

It can be noted from Figure, 1 that, in all the species under study, maximum quantity of DNA was isolated from legs as compared to other body parts. We support our results with the facts that, legs are composed of only muscle cells and no enzyme producing or secreting cells. On contrast, in thorax and abdomen, cells which secrete enzymes are also present. These enzymes can inhibit the DNA isolation process or can also degrade the DNA.

Apart from this, legs have an additive advantage that they can be easily removed and even a single leg is sufficient to extract DNA for further molecular processes. Thus, rest of the body can be preserved as voucher and only leg can be removed for molecular assessment. Similar results were reported by Shere-Kharwar et al., 2012 in sphingid moths.

Thus, we propose that legs are excellent source of tissue for DNA isolation not only with respect to the quantity of DNA isolated from them but also in terms of simple and non tedious tissue extraction and maintaining the voucher specimen for future reference.

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